UAMS
UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Department of Neurobiology and Developmental Sciences
Center for Translational Neuroscience
College of Medicine

Neuronal Signals - NBDS 5161
Session 1: Design of an electrophysiology setup

Abdallah HAYAR

Lectures can be downloaded from
http://hayar.net/NBDS5161

Updated Tentative Schedule for Neuronal Signals (NBDS 5161)
One Credit–Hour, Summer 2010
Location: Biomedical Research Building II, 6th floor, conference room,
Time: 9:00 -10:20 am

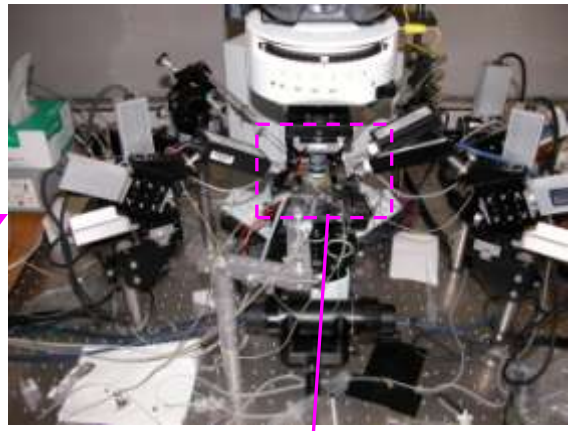
Session	Day	Date	Topic	Instructor
1	Tue	6/1	Design of an electrophysiology setup	Hayar
2	Thu	6/3	Neural population recordings	Hayar
3	Thu	6/10	Single cell recordings	Hayar
4	Fri	6/11	Analyzing synaptic activity	Hayar
5	Mon	6/14	Data acquisition and analysis	Hayar
6	Wed	6/16	Analyzing and plotting data using OriginLab	Hayar
7	Fri	6/18	Detecting electrophysiological events	Hayar
8	Mon	6/21	Writing algorithms in OriginLab®	Hayar
9	Wed	6/23	Imaging neuronal activity	Hayar
10	Fri	6/25	Laboratory demonstration of an electrophysiology and imaging experiment	Hayar
11	Fri	7/9	Article presentation I: Electrophysiology	Hayar
12	Mon	7/12	Article presentation II: Imaging	Hayar
13	Wed	7/14	Exam and students' survey about the course	Hayar

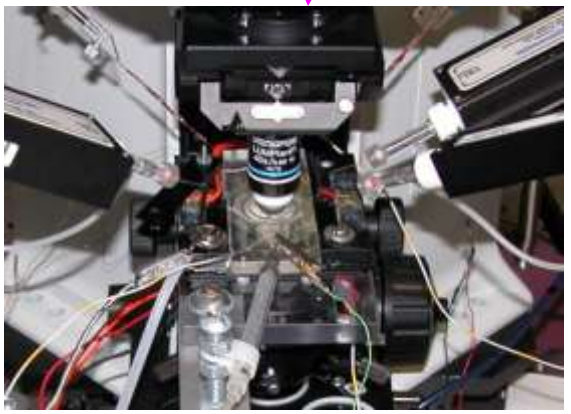
Student List

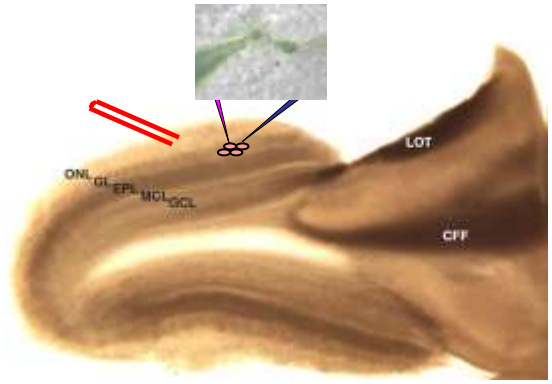
	Name	E-mail	Regular/Auditor	Department	Position
1	Simon, Christen	CSimon@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
2	Kezunovic, Nebojsa	NKezunovic@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
3	Hyde, James R	JRHyde@uams.edu	Regular (form signed)	Neurobiology & Developmental Sciences	Graduate Neurobiology – Mentor: Dr. Garcia-Rill
4	Yadlapalli, Krishnapraveen	KYadlapalli@uams.edu	Regular (form signed)	Pediatrics	Research Technologist – Mentor: Dr. Alchaer
5	Pathan, Asif	APATHAN@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – Mentor: Dr. Rusch
6	Kharade, Sujay	SKHARADE@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Pharmacology – 4th year - Mentor: Dr. Rusch
7	Howell, Matthew	MHOWELL2@uams.edu	Regular (form signed)	Pharmacology & Toxicology	Graduate Interdisciplinary Toxicology - 3rd year - Mentor: Dr. Gottschall
8	Atcherson, Samuel R	SRAtcherson@uams.edu	Auditor (form signed)	Audiology & Speech Pathology	Assistant Professor
9	Detweiler, Neil D	NDDETWEILER@uams.edu	Auditor	Pharmacology & Toxicology	Graduate Pharmacology – 1st year
10	Thakali, Keshari M	KMThakali@uams.edu	Possible auditor	Pharmacology & Toxicology	Postdoctoral Fellow – Mentor: Dr. Rusch
11	Boursoulian, Feras	FBoursoulian@uams.edu	Possible auditor	Neurobiology & Developmental Sciences	Postdoctoral Fellow – Mentor: Dr. Hayar
12	Steele, James S	JSSTEELE@uams.edu	Possible auditor	College of Medicine	Medical Student – 1st Year – Mentor: Dr. Hayar
13	Beck, Paige B	PBBeck@uams.edu	Possible auditor	College of Medicine	Medical Student – 2nd Year- Mentor: Dr. Garcia-Rill

Electrophysiological and imaging setup

Electrophysiological and imaging setup: Brain slices







- Olfactory bulb slices (400 µm thick) were cut from young rats (21- 30 days).
- Single and paired whole-cell current and voltage clamp recordings were made at 30°C; drugs applied by perfusion.
- Recorded neurons filled with Lucifer Yellow and biocytin and their morphology was reconstructed.
- Olfactory nerve (ON) electrical stimulation.

In vivo recordings

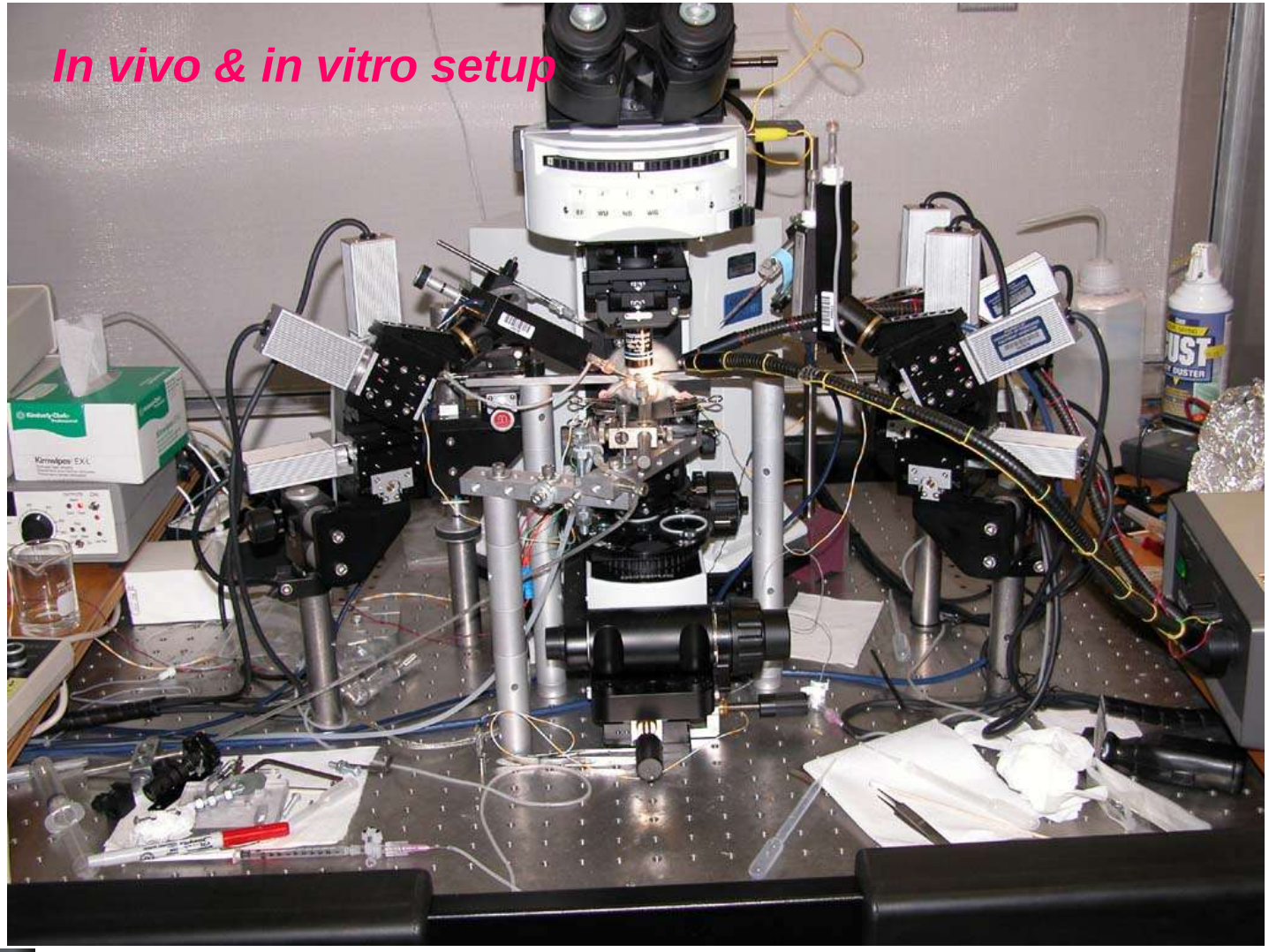
-Single and multiunit

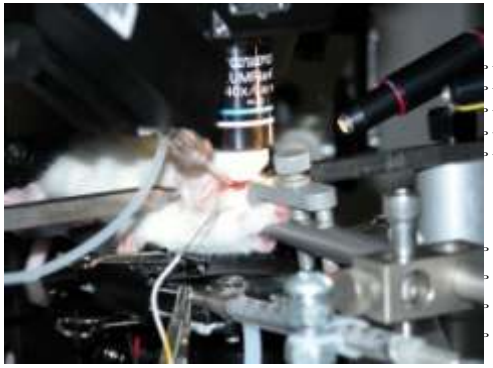
-Extracellular / patch clamp

-Anesthetized vs. awake animal

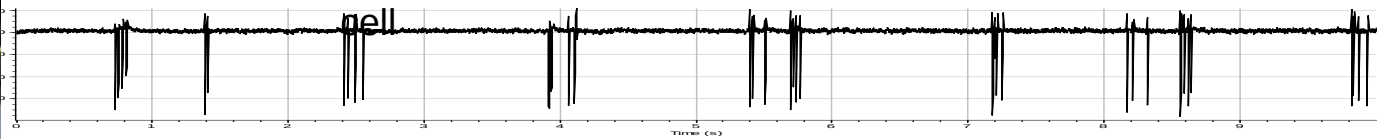
-Calcium / voltage-sensitive dye imaging

-Odor stimulation

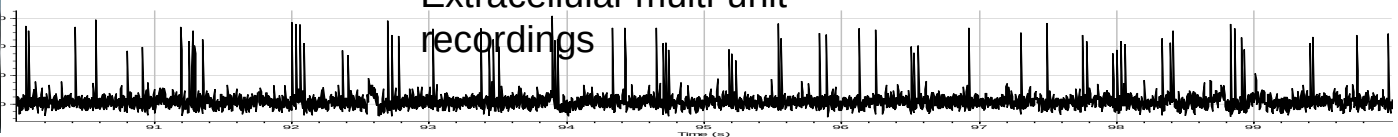




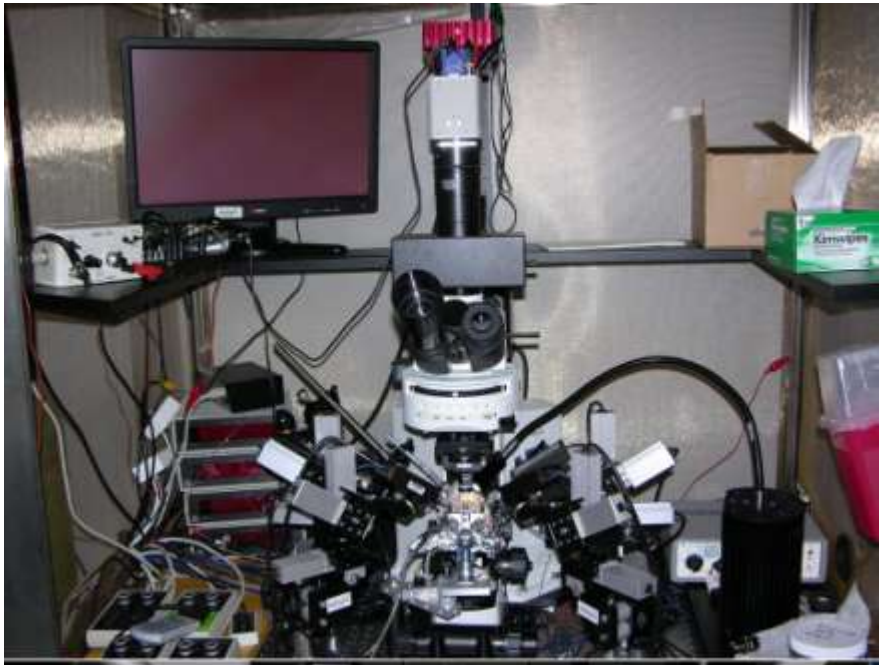
Extracellular recording from a bursting cell

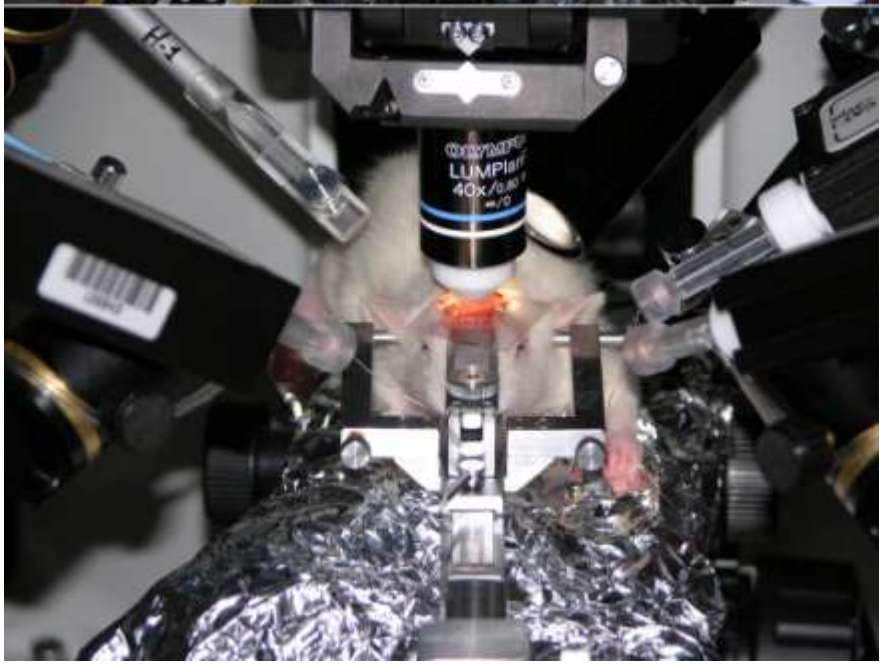


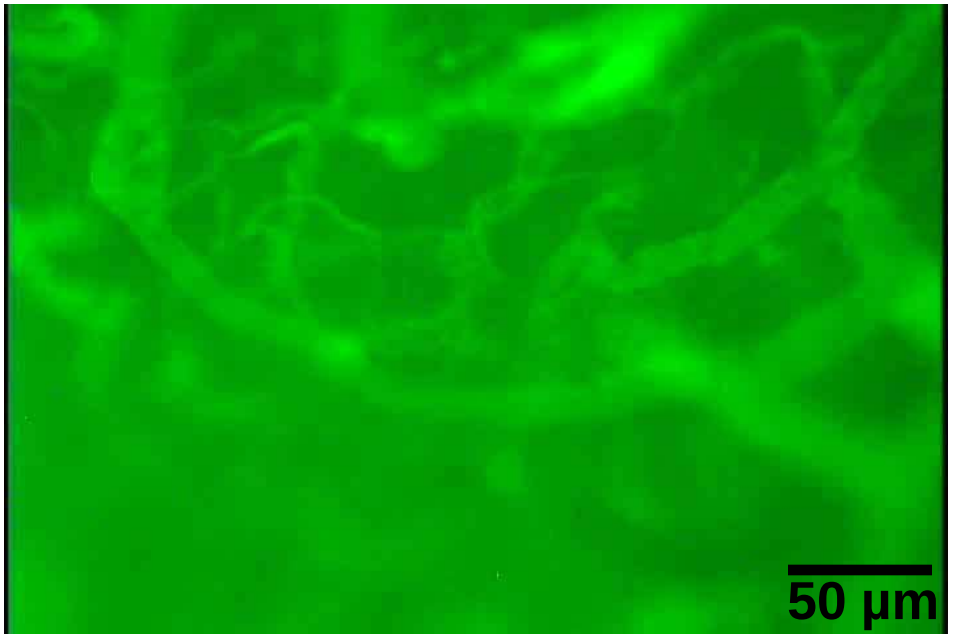
Extracellular multi-unit recordings



Video microscopy of cerebral blood flow

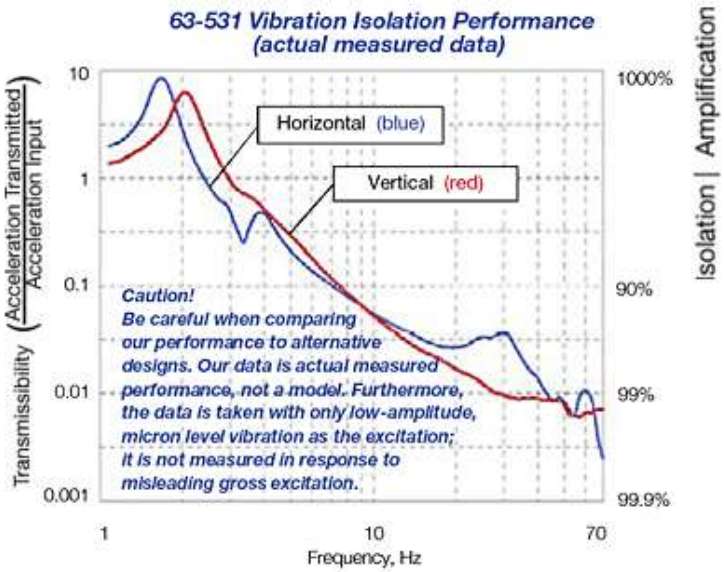






63-543
30"x48"-75x120 cm Isolator with 4" CleanTop II, 1/4-20 on 1in. spacing
$3,755.00

Gimbal Piston™ Isolators



83-014-01
Set of 4 retractable caster
$505.00

Faraday cage

81-333-04
30" x 48" x 40" Type II Faraday cage
$1,385.00

81-335-04
U-shaped hanging shelf for 48" Faraday cage
$240.00

81-332-04
8" x 30" 2 Sliding shelves for Faraday cage
$110.00

81-322-04
Perimeter enclosure for 4” top
$260.00

81-303-02
Armrest pad for Perimeter Enclosure or Faraday cage
$45.00

Observation Tube : $2,578

Illuminator for fluorescence: $2,442

Mercury Lamphouse: $1229

2 Eyepieces: $348

Density filter 12%: $66

Each filter cube: $800

100 W Mercury Power Supply: $1553

Dual objective nosepiece: $1305

40 X Objective: $2079

Arm for nosepiece: $592

Condenser: $1,260

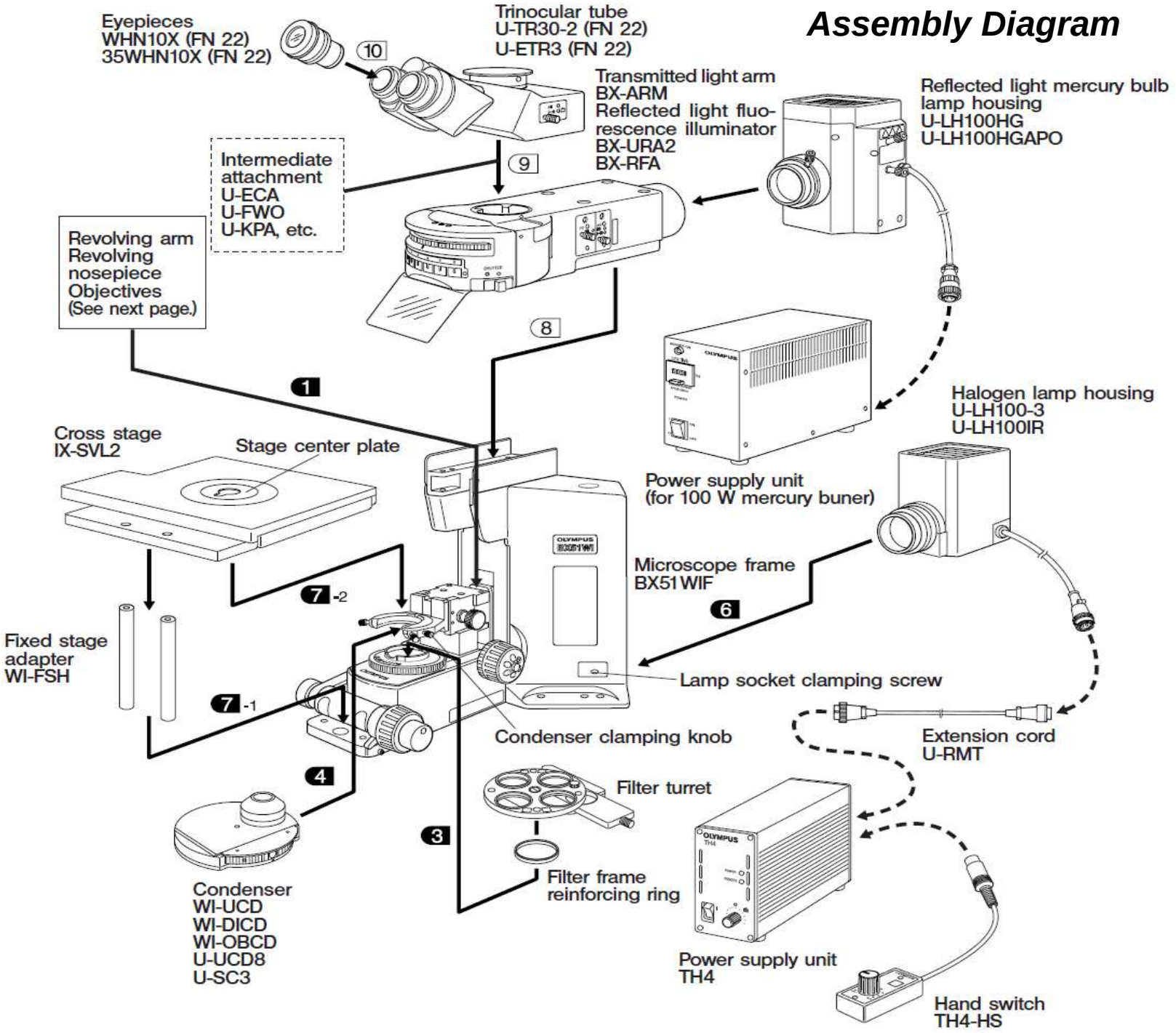
Halogen lamphouse: $539

Halogen bulb: $70

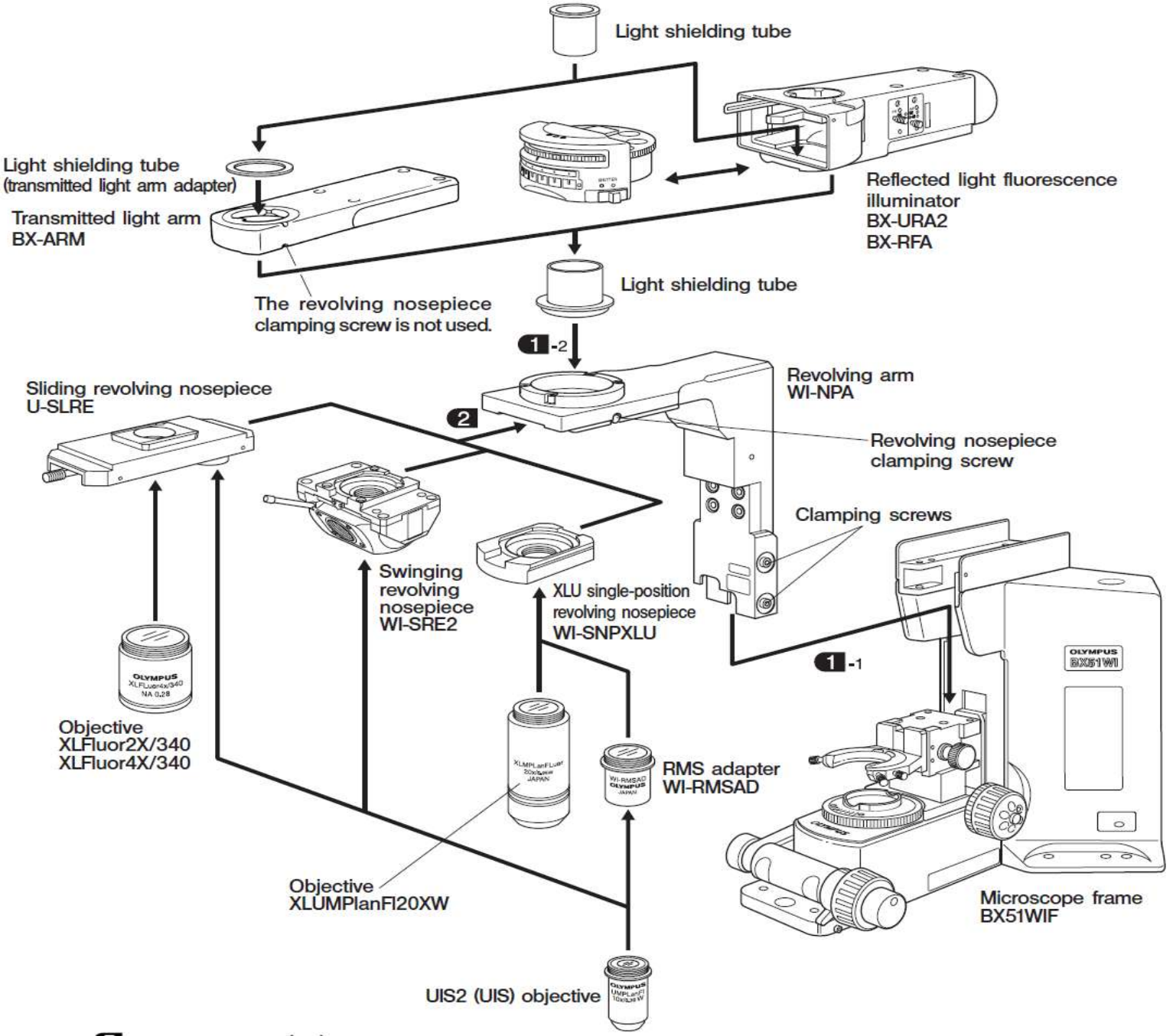
Frame: $4,513

0-12 V transformer: $516

Assembly Diagram



Assembly: Revolving Arm, Revolving Nosepiece, Objectives



Mercury Arc lamps

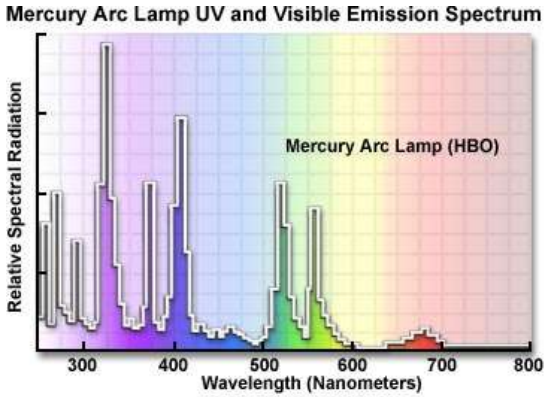
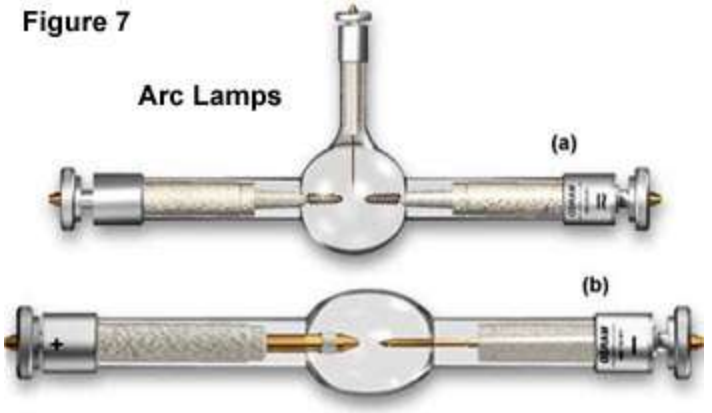


Figure 8



Tungsten-halogen lamps

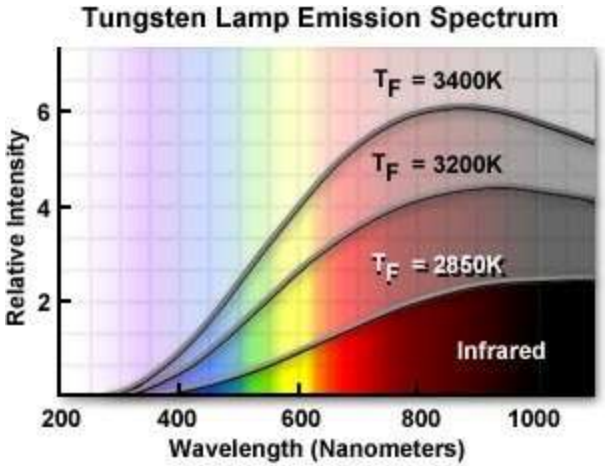
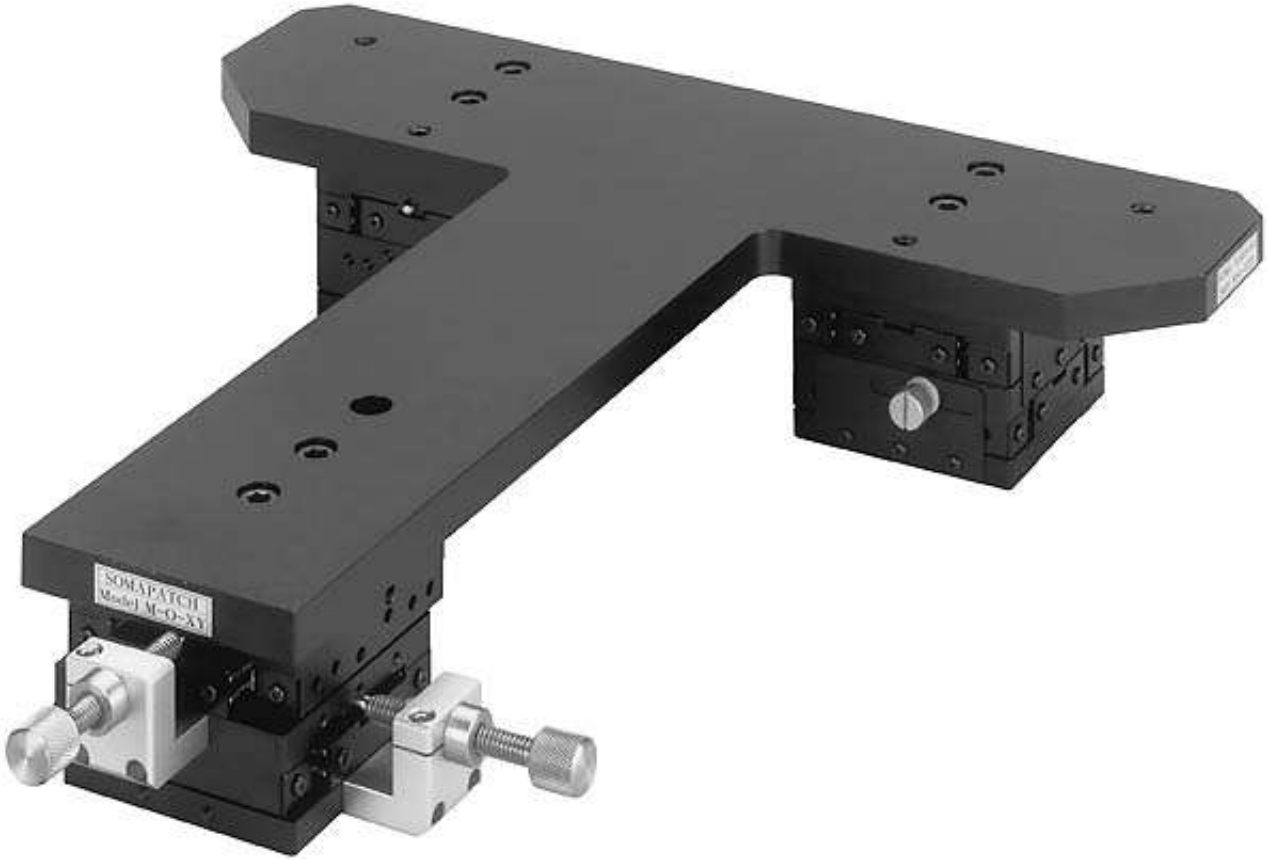


Figure 2

Figure 3

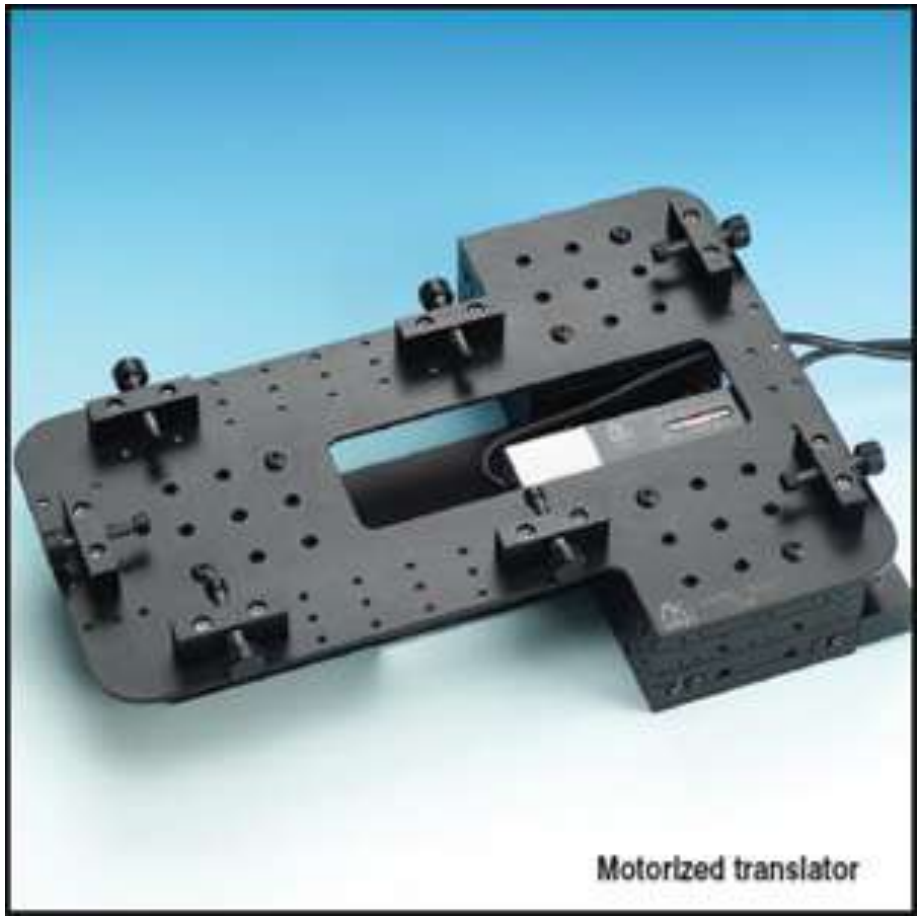
X-Y Translator

SOMAPATCH-O-XY XY translator for Olympus BX51WI-2950$



Siskiyou: Adjustment Screws

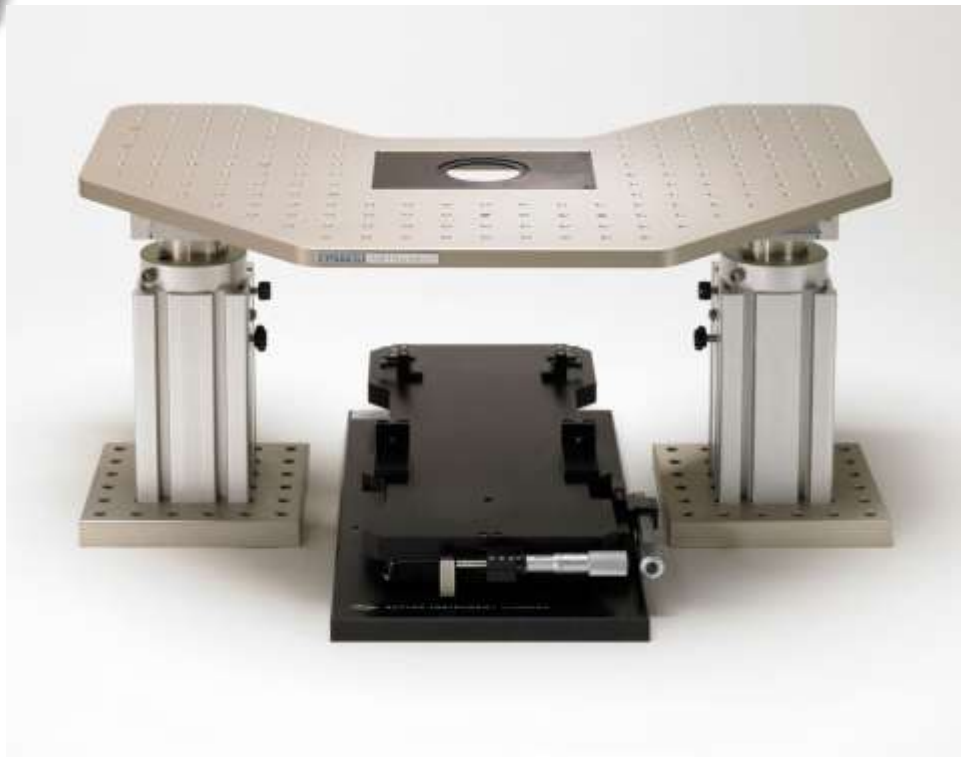
siskiyou.com $3500



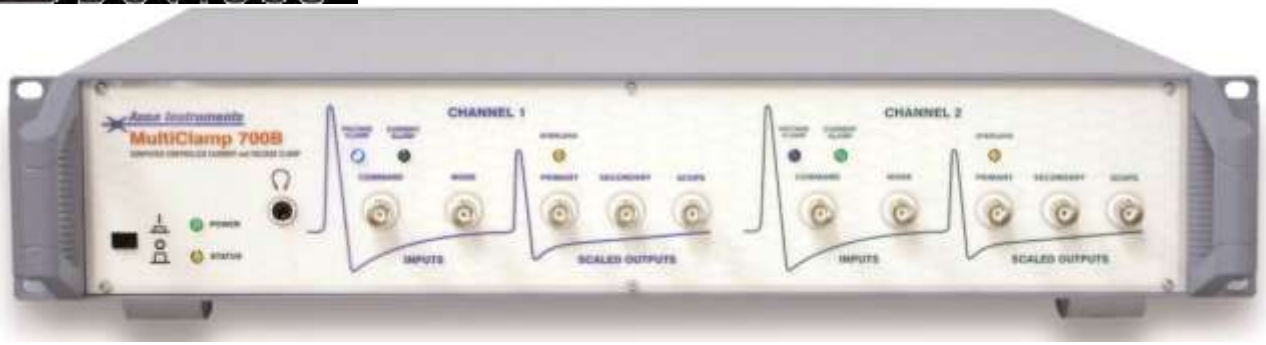
■ 20-, 40-, 80-, or 100-pitch drive screws
■ Compatible with fixed stage microscopes
■ Full crossed roller stage support
Performance Specifications
Maximum load 200 lbs, centered Travel / axis 1.0 inch [25 mm] Minimum controllable motion Motorized Backlash ≤ 5 µm Point to point accuracy ± 2 µm

Sutter Instruments MT-500/Y51

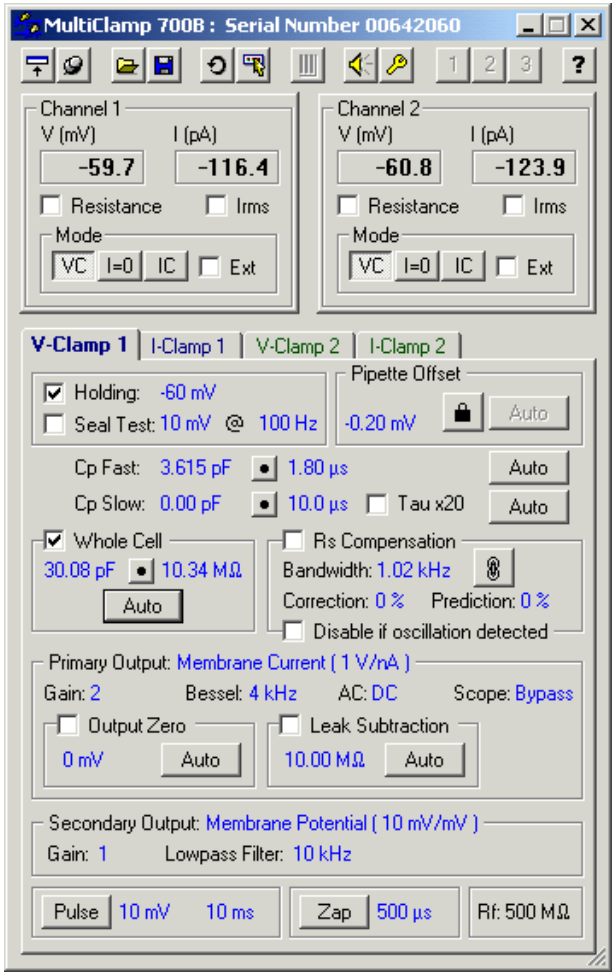
X-Y translator for Olympus BX51WI **$ 3,900**



MultiClamp 700B: $14,482



Software Control



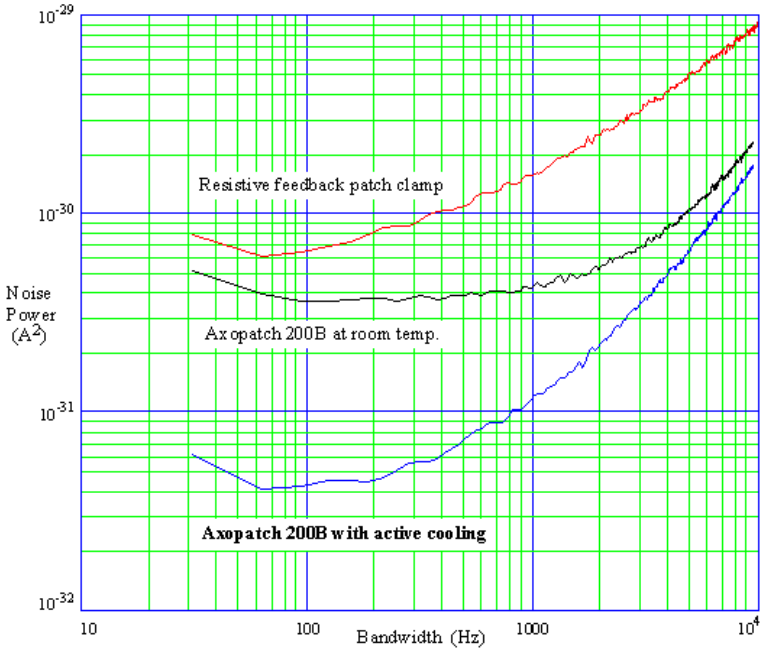
The MultiClamp 700B is useful for

- Intracellular or extracellular recording
- High-speed Current Clamp (sharp-electrode or field potentials)
- Patch Clamp (whole-cell, macro-patch or excised patch)
- Voltammetry / Amperometry
- Ion-selective measurement
- Bilayer recording

MultiClamp 700B Commander, a program that runs on a host computer (PC and Macintosh) and communicates with the amplifier via a USB cable.

Axopatch 200B: $12,398

The **Axopatch 200B** patch clamp offers the lowest-noise patch-clamp amplifier technology.
Noise is 0.145 pA rms below 10 kHz bandwidth with a pipette holder attached.
This noise performance is achieved in part by cooling the input field-effect transistors inside the headstage to well below 0° C.



Noise spectra from patch-clamp amplifiers showing the noise contribution of the amplifier. Top trace, a resistive headstage; Middle trace, Axopatch 200B at room temperature; Bottom trace, Axopatch 200B with circuitry cooled to -20° C. Even at room temperature, the noise performance of the capacitor-feedback amplifier is clearly better than the resistor-feedback amplifier. Cooling dramatically reduces the noise even further, for the lowest noise ever.

Microelectrode Amplifier Current Clamp and Voltage Clamp

Axoclamp 2B

Multiclamp 900A: $9,676

I-Clamp: two independent bridge amplifiers for voltage measurements
DCC: discontinuous current clamp for accurate voltage measurements, even when electrode resistance changes
TEVC: high-compliance two-electrode voltage clamp for oocytes and mammalian cells
dSEVC: discontinuous single-electrode voltage clamp for small cells with large currents
HVIC: high-voltage current clamp for extracellular applications such as iontophoresis

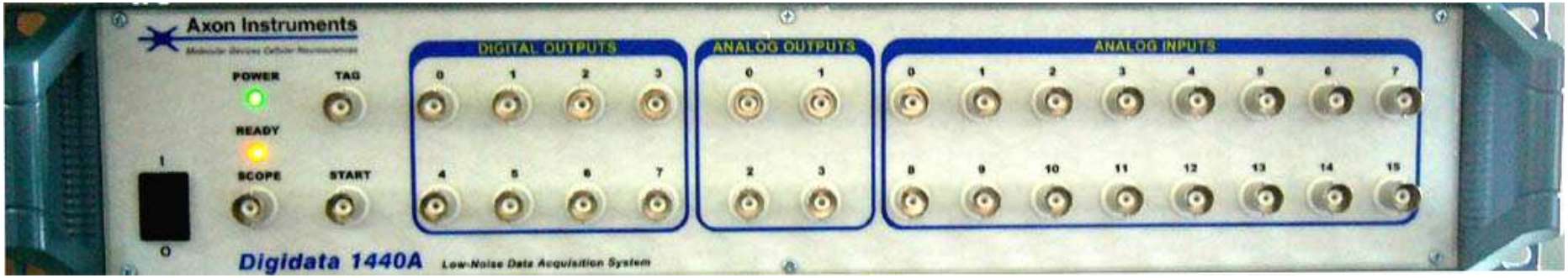
Note that this amplifier does not have continuous single-electrode voltage clamp mode.

Software Control

SoftPanel

Applications	Axoclamp 900A	MultiClamp 700B	Axopatch 200B
Voltage Recordings	2 ch.	2 ch.	1 ch.
Current Passing	2 ch.	2 ch.	1 ch.
Bridge Mode	2 ch.	2 ch.	
TEVC	✓		
Discontinuous Current Clamp	✓		
Discontinuous SEVC	✓		
cSEVC or Whole-cell		✓	✓
Single-Channel Recording		✓	✓
Ion-Selective Electrodes	✓	✓	
Ionophoresis	✓	✓	
Voltammetry		✓	✓
Features			
Rs Compensation	✓	✓	✓
Pipette Cap. Comp.	✓	✓	✓
Whole-cell Cap. Comp.		✓	✓
Low-pass Filters	✓	✓	✓
Seal Test	✓	✓	✓
Step-Voltage Generator	✓	✓	
Audio Monitor	✓	✓	
RMS Noise Monitor		✓	✓
Gain Telegraph	✓	✓	✓
Frequency Telegraph	✓	✓	✓
Capacitance Telegraph		✓	✓
Capacitative Feedback (integrating headstage)			✓
Zap, Buzz or Clear	✓	✓	✓
Blank	✓		✓
Track	✓		✓
Bridge Balance	✓	✓	✓

Digidata 1440A: Low-noise Data Acquisition System: $5,420.00



USB 2.0 interface; no need for a PC card.

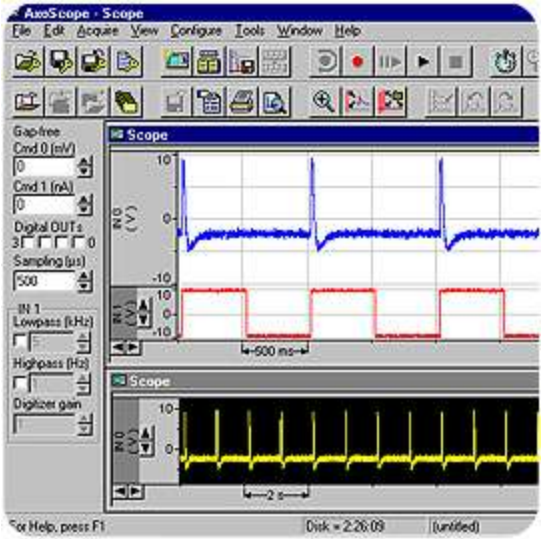
Number of Analog Inputs: 16 channels
Sampling rate up to 250 kHz/channel X 16 input channels = 4 MHz = 4,000,000 samples/sec x 16 bits/sample = 64 Mbps
Input Range: -10 to +10 Volts
Resolution 16 bits/sample; 20 volts/ 2^{16} = 20/65536= 0.3 mV

Number of Analog Outputs: 4 channels
Output Range: -10 to +10 Volts
Resolution 16 bits;

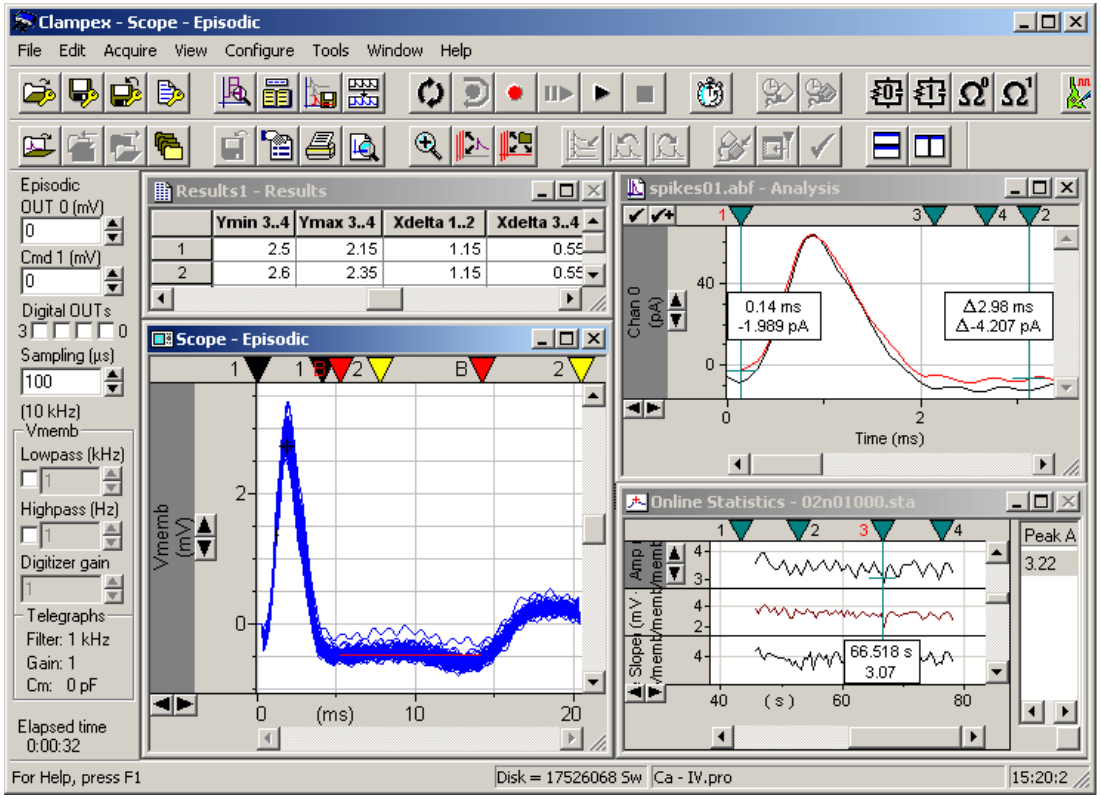
Number of Digital Inputs: 8 channels
Number of Digital Outputs: 16 channels
Dedicated digital output to trigger devices such as oscilloscopes
Trigger inputs to start acquisition and to tag data

Digitizer and channel crosstalk noise < ±1 mV average p-p at 10 kHz.

Basic Software: AxoScope



Pclamp 10: Conventional Electrophysiology Data Acquisition and Analysis Software $4,902.00



Three separate programs are included:

AxoScope 10 provides a convenient way to produce background recordings with the included MiniDigi 2-channel digitizer*. You can monitor cells during intra-sweep periods, or create an overview of the entire day's activities including voice tags.

Clampex 10 expands the range and quality of your data acquisition experiments. These improvements should prove useful for a wide variety of applications, allowing more flexibility in your experimental protocols.

Clampfit 10 is a powerful solution for analyzing, graphing and layout of all of your Clampex and AxoScope data. Clampfit includes an extensive array of filtering and fitting routines. Functionality includes I-V graphs, power spectrums, and special "linked data views" for threshold (AP), template (minis) and single-channel modes of event detection and analysis.

Tektronix TDS2014 standard oscilloscope; digital, 100 MHZ, 1 GS/S, 4-CH, 2,150$

Digital real-time sampling Accurately capture signals with at least 5x oversampling on all channels, all the time.

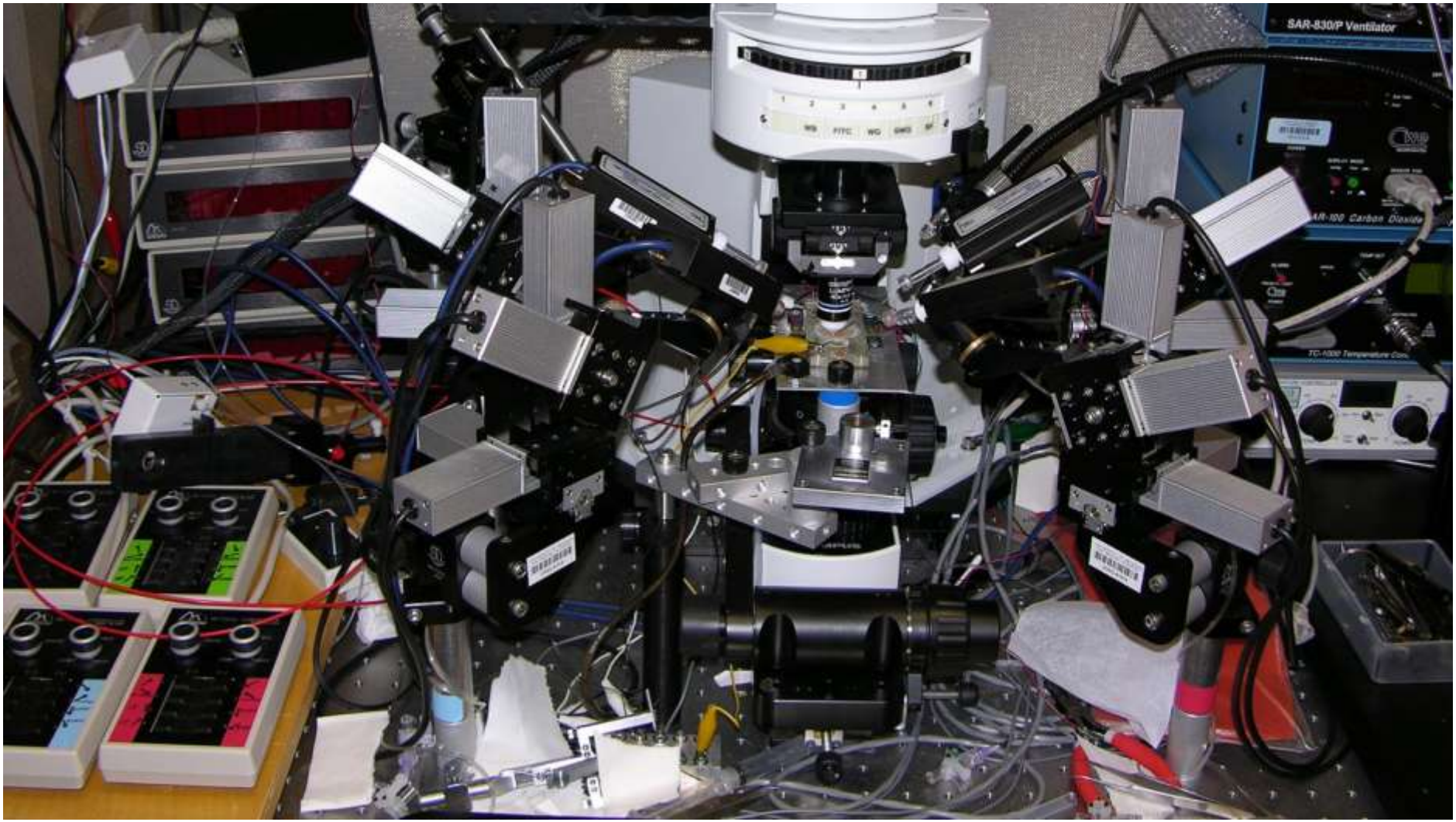
Dedicated front-panel controls Spend less time learning and more time on your task with front-panel controls.

Front-panel USB host port Quickly store and transfer your waveforms.

USB plug-and-play PC connectivity Easily document and analyze your results with NI LabVIEW

SignalExpress™ TE and Tektronix OpenChoice® Desktop software.

Direct print capability
Lifetime Warranty
Lightweight Just 4.4 lbs (2 kg)


SAR-830/P Ventilator

Micromanipulators Motorized, Post Mount 4 Axis

Model; MX7500L

- **1.7 mm/second rapid positioning**
- **Axial approach on 4th axis**
- **Repeating robe holder**

Performance Specifications

- 1.7 mm/second rapid positioning
- Axial approach on 4th axis
- Repeating probe holder

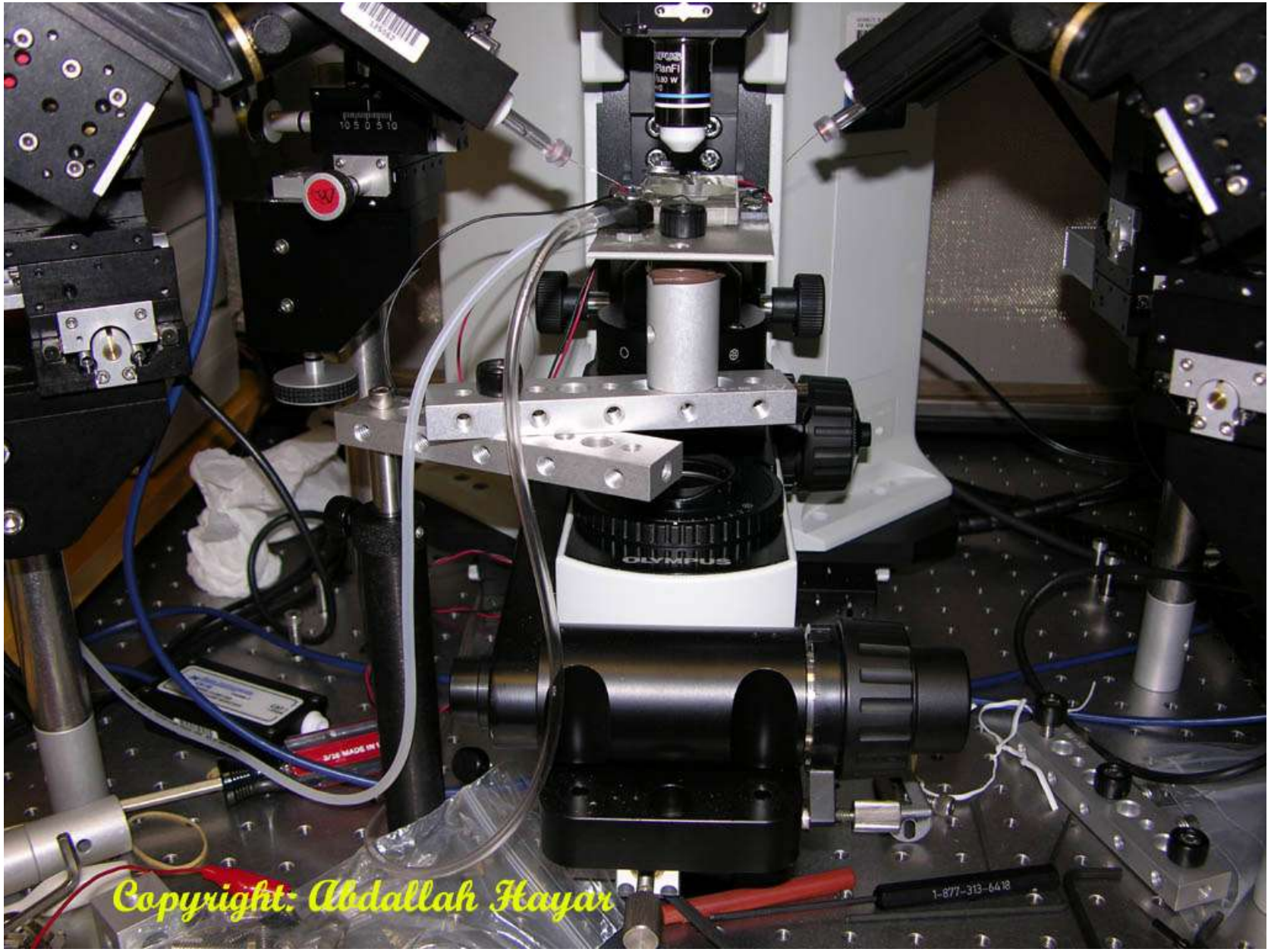
Performance Specifications
Maximum load 2 lbs
Travel / axis 0.80 inch (20 mm)
Minimum controllable motion 0.1 µm
Backlash ≤ 5 µm
Point to point accuracy ± 2 µm

- Economical DC controller
- Variable high resolution speed control
- Preset rapid and medium speed setting

Performance Specifications
Minimum controllable motion 0.2 µm

- User set target point
- 4-axis intuitive dial control
- Remote micrometer control
- High and low speed settings

Performance Specifications
Minimum controllable motion 0.2 µm
Maximum speed 1.7 mm/sec



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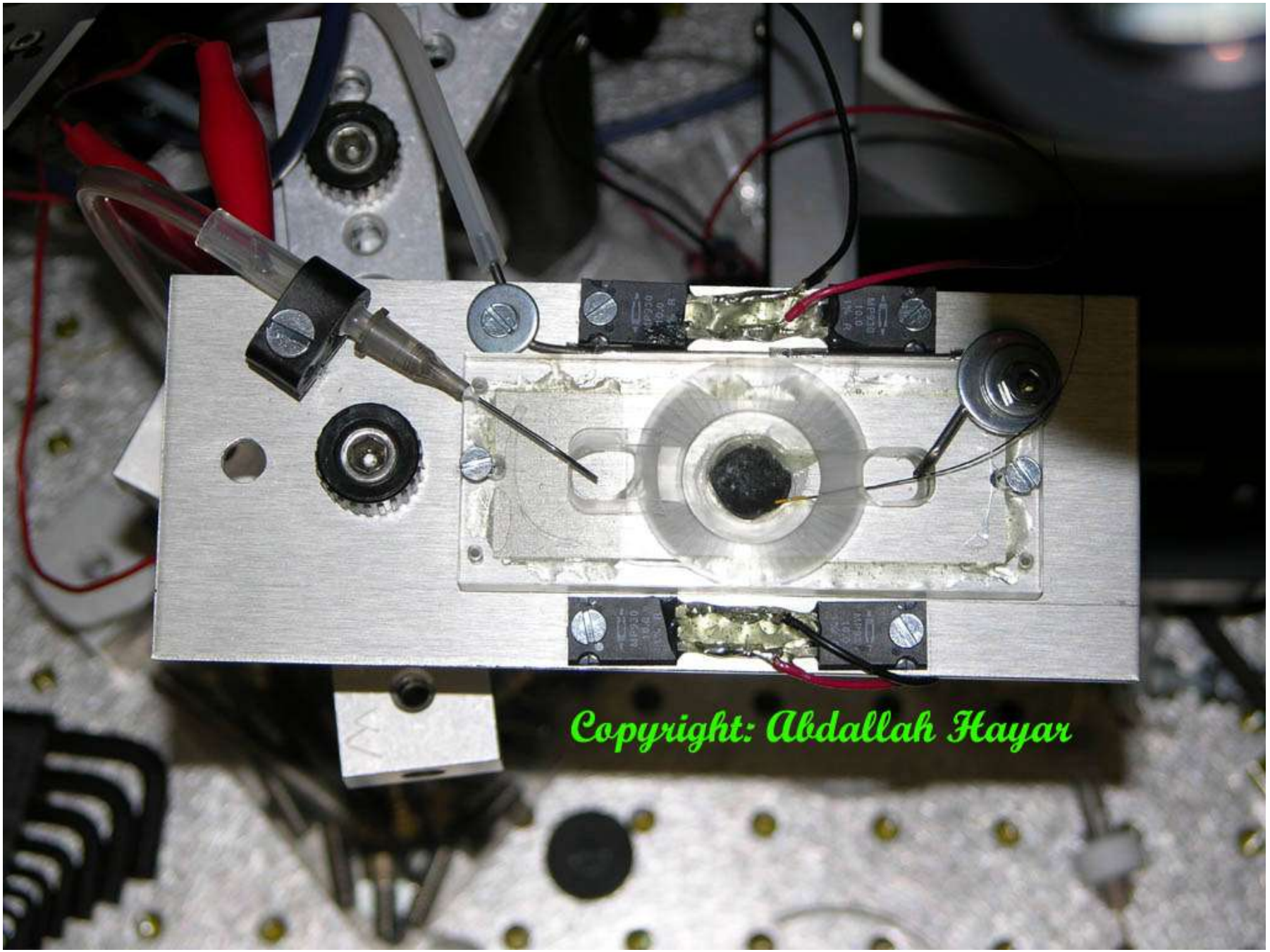
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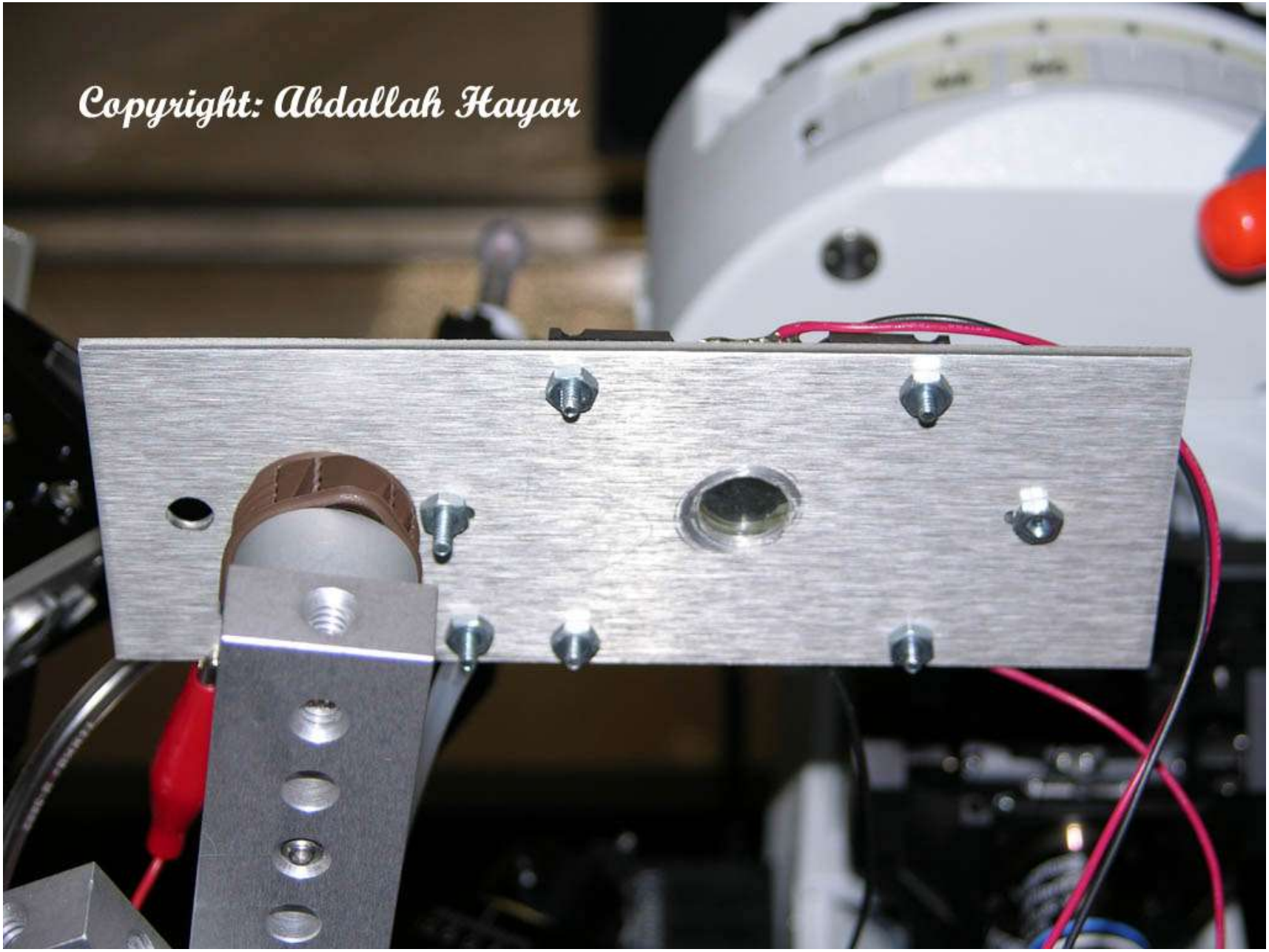
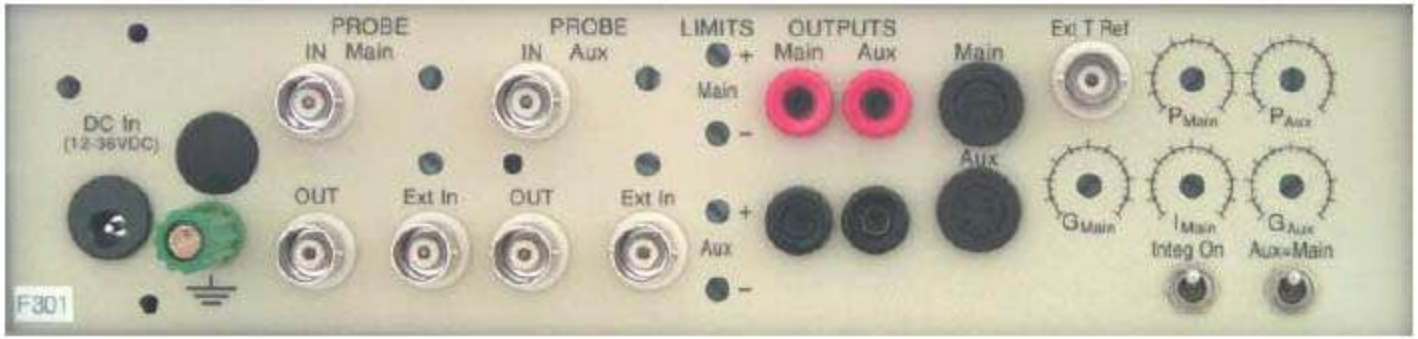

Copyright: Abdallah Hayar

Analog TC2BIP 2/3Ch Bipolar Temp controller: $1,645



BT-xx

$175

HI-xx

$105

TH-10Km

HLS-xx

12 Volt 18 Ah Sealed Lead Acid Battery $34.95

Specifications:

BM Part #:	SLA-12V18
Voltage:	12 Volt
Capacity:	18 Ah
Type:	Sealed Lead Acid Battery
Warranty:	1 Year
Shipping Weight:	15.00 Pounds
Length:	7.13"
Width:	2.99"
Height:	6.57"

Battery Defender Pro 12 Volt 3 Amp Battery Charger $36.95

BM Part #:	ACC-1206WB
Voltage:	12 Volt
Capacity:	3 Amps
Warranty:	1 Year

Features:

De-sulfation of battery
Short circuit protection
Reverse polarity protection
No current drain
Maintain full charge & no overcharging

Cole-Parmer: Masterflex Peristaltic Pump: $1442

Variable speed modular drive,
1-100 rpm, 115V, $690,
Cat# EW-07553-80

4-channel, 8-roller cartridge
pump head. $300,
Cat# EW-07519-20

4 small cartridges for 07519-20
and -25 pump heads. $74 X4
Cat# EW-07519-85

- Flow rate: 0.06 to 580 mL/min depending on tubing size and drive rpm; achieve lower flow rates with cartridge or multichannel pump heads and microbore tubing.
- 6-ft (1.8-m) cable connects motor and controller—place motor and controller where convenient.
- Reversible motor lets you purge tubing before or after pumping, or pump in either direction.
- Separate single-turn potentiometer and on/off/reverse switch with inertia center.

Replacement brushes for
Masterflex drive 07553, $26,
Cat# EW-07520-04

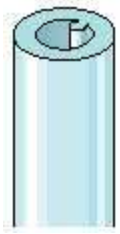
BioPharm Plus platinum-cured silicone pump tubing, L/S® 13, 25 ft. (package) $59,
Cat# EW-96440-13

BioPharm Plus platinum-cured silicone pump tubing, L/S® 14, 25 ft. (package) $71,
Cat# EW-96440-14

P-97 Flaming/Brown Micropipette Puller: $ 7,090

SUTTER INSTRUMENT

Environmental chamber.
Programmable air pressure.
Memory storage for up to 100 programs.
Write protection and date stamp for each program.
Two symmetrical pipettes with each pull.
Preprogrammed sample programs for intracellular and patch pipettes. Special programming on request.
Ramp test—self test for establishing program heat settings when a new filament or glass is introduced.
Vacuum fluorescent display.
Internal memory test.
Constant current power supply for filament and pull solenoid.
Looping pull cycle for fabrication of patch type micropipettes.
Self-contained air supply with filtration system and humidity control.
Consistent and reliable electrodes with tip diameters less than 0.1µm.
Control over the time and pressure at which the air is delivered.
Optimized velocity sensing circuit for maximized sensitivity and reproducibility.
Quality control, SEM photograph of a tip pulled with each puller; criterion is tip measurement less than 0.1µm and typically is ~0.06µm.



WPI: Cat# Order # 1B150F-3
3 in. (76 mm)
1.5 / 0.84 OD/ID (mm)
Filament
225 per vial, Price $45

Leica VT1000P Manual Vibrating Microtome: $4,845

Subzero™ Freezing Microtome Stage: $2,995

General rules for brain
Bath – cold (2 – 6 degrees C.)
Speed – slow (setting 1-2 or less)
Amplitude – High (8 +/-)
Blade Angle – Steep (25 degrees +/-)

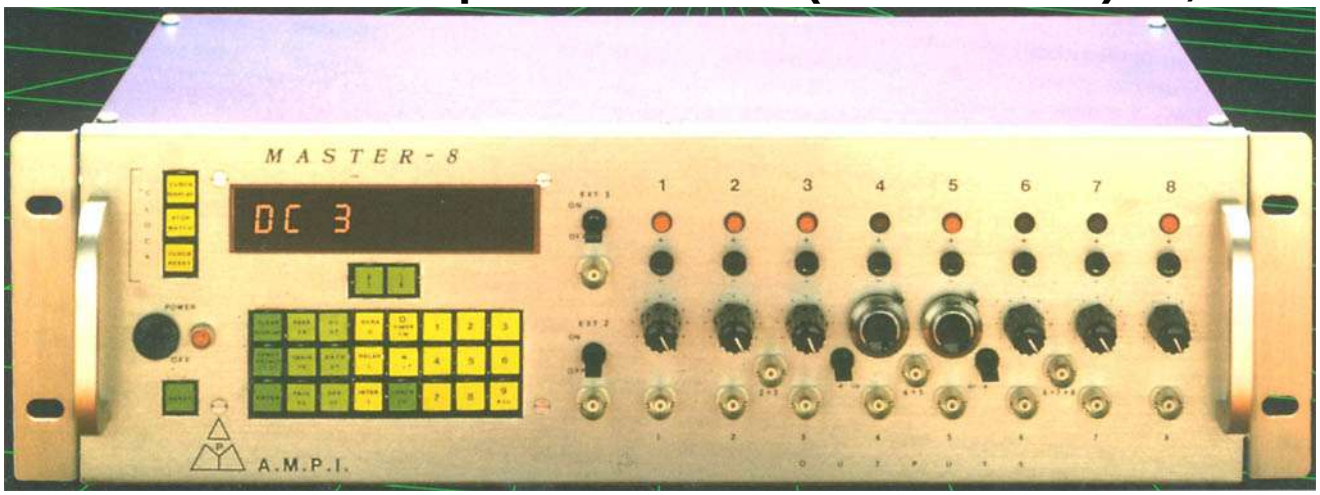
The principle of the VIBRATOME is a Vibrating blade intersecting the specimen underneath the surface of a liquid bath, which lubricates the cut. Next there are 5 parameters under the control of the operator:

1. Nature of the bath (usually a physiologically compatible buffer).
2. Temperature of the bath (the softer the specimen, the colder the bath should be).
3. Speed (the softer the specimen, the slower the speed).
4. Amplitude (the softer the specimen, the higher the amplitude).
5. Blade Angle (the softer the specimen, the steeper angle of attack required).

Embedding when necessary (i.e. Fresh brain at 20 microns). Do not use agar. Use 2-5% neutral agarose. Trial and error on the exact concentration. The trick is to match the density of the agarose with that of your specimen.

Vibratome makes no claim for less than 20-micron sections using a standard razor blade. However, many people have successfully cut firmer specimens at 10 microns using a glass or sapphire knife.

Master-8 cp is an 8-channel programmable pulse stimulator with computer interface (serial + USB) $3,950



All eight channels can be operated independently, or synchronized to produce complex patterns of pulses. Use some channels to stimulate your preparation, while using other channels to trigger other instruments (oscilloscope, PC, etc.) in synchronization with the stimulation.

Each channel can operate in any of the following modes.
Free Run: The channel generates pulses continuously, Time rate and duration of the pulses can be set individually for each channel.
Trig: After triggering (manually by one keystroke, internally from other channels or externally from other devices) the channel delivers a single pulse at the preset delay and duration times.
Train: After triggering, the channel delivers a preset number of pulses with the set interval and duration times. Master-8 can automatically display the serial number of the pulse in the train for one channel.

123 Uziel St., P.O.B. 16477
Jerusalem 91163, ISRAEL
phone: +972 2 643-9338
fax: +972 2 643-1833
e-mail: info@ampi.co.il
www.ampi.co.il
A.M.P. INSTRUMENTS LTD

ISO-Flex: Stimulus Isolator: $680

Flexibility
ISO-Flex is a flexible stimulus isolator because
- It is optically isolated.
- You can switch the unit to constant current or constant voltage by the CURRENT/VOLTAGE switch.

Specifications:
Input: 5V-10V
Voltage output: 0-Veffective.
Veffective = (Vbattery-3) Volt.
Current output: 0-10mA.
The output is divided into 3 ranges.

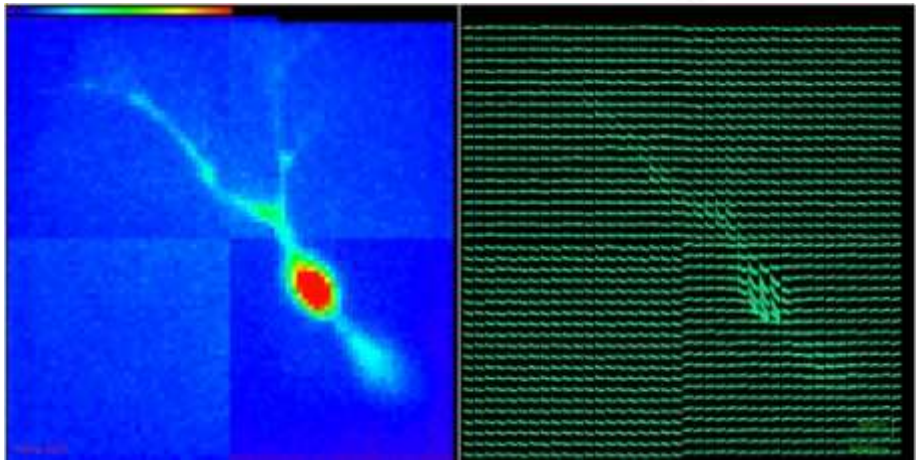
ISO-Flex is supplied with 2 batteries:
- a standard 9V battery in the ISO-Flex box and
- a 90V battery in the battery housing.

The 9V battery is used for operating the isolator.
The 90V battery is used to drive the output pulses.

REDSHIRT IMAGING

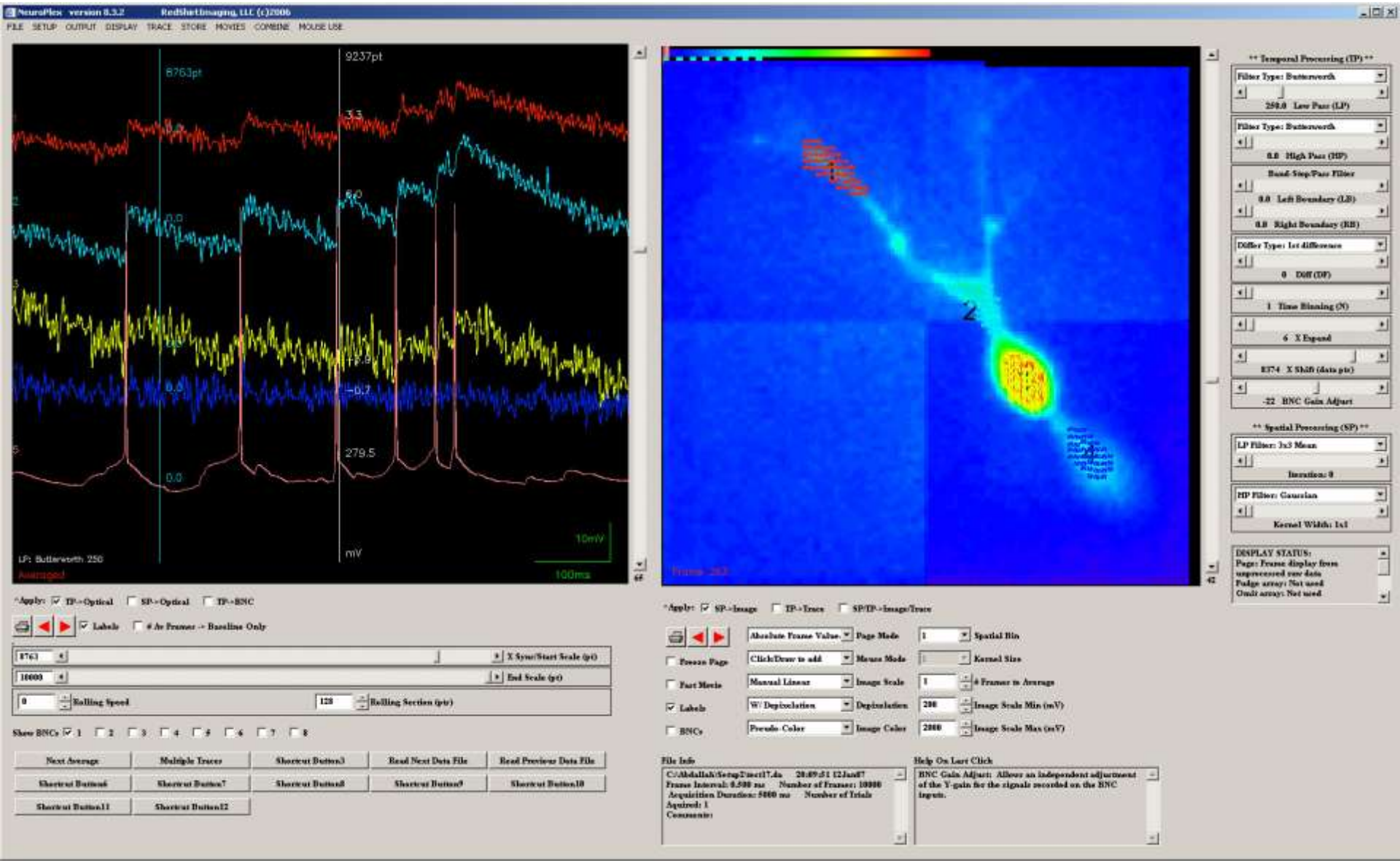
NEURO CCD-SMQ

is capable of recording action potential propagation in individual neurons with outstanding temporal resolution:
80 x 80 pixels: 2000 frames/sec
3 x 3 bin: 5000 frames/sec



1 frame = 80 x 80 pixels = 6400 pixels or channels at 14 bits
+ 8 channels of electrical recording
2000 frames /sec = 25 Mbytes/sec
1 CD =700 Mbytes = 30 sec of data
1 DVD= 4.3 Gbytes = 3 min of data

Fast optical recordings will help resolve the time of occurrence of action potentials



Anatomy of a computer

Motherboard: ASUS P5Q Deluxe LGA 775 Intel P45 Intel $194.99

Video Card: MSI RX3870X2-T2D1G OC Radeon HD 3870 X2 1GB, $209

Power Supply: PC Power and Cooling S75QB 750W ATX12V, $123

Processor: Intel Core 2 Quad Q9550 Yorkfield 2.83GHz, $278.99

RAM: G.SKILL 4GB (2x 2GB) 240-Pin DDR2 SDRAM DDR2 1066, $109.99

DVD drive: SAMSUNG Black 22X DVD+R 8X DVD+RW 16X DVD+R, $26.99

Operating System: Microsoft Windows Vista Home Premium SP1 64-bit, $99.99

Case Fan: Antec 75009 92mm, $14.99

Processor cooler: ARCTIC COOLING Freezer 7 Pro Rev.2 92mm Fluid Dynamic CPU Cooler, $33.94

Hard Disk: Western Digital Caviar Black WD1001FALS 1TB 7200 RPM 32MB Cache SATA 3.0Gb/s, $99.99

Computer Case: Antec Solo Black/Silver Steel ATX Mid Tower, $94.99

Keyboard & Mouse: Logitech S 510, 104 Normal Keys RF Wireless Slim Cordless Desktop $49.99

Monitor: SAMSUNG 2443BWT-1 Black 24" 5ms Widescreen Height Swivel tilt & pivot Adjustable LCD Monitor 300 cd/m2 DC 20000:1, Resolution 1920 x 1200, $399.99

Router: D-Link DIR-655 IEEE 802.3/3u, IEEE 802.11g, IEEE802.11n Draft 2 Xtreme N Gigabit Wireless Router, $89.99

U.S. Robotics USR5637 56Kbps USB High-performance V.92 modem, $44.99

Pinnacle Dazzle Video Creator Plus white Box 82301006461 USB 2.0 Interface, $34.99

D-Link DUB-H7 7-Port USB Hub, $28.99

LACIE Little Disk 500GB USB 2.0 External Hard Drive 301841N, $104.99

Kingston 16GB Flash Drive (USB2.0 Portable) Model DTMS/16GB, $44.86